12

RELATIVE ABUNDANCE OF SERUM PROTEINS IN ALBINO RATS AT DIFFERENT AGES

BY

IKUZO TOYAMA

(From the Wistar Institute of Anatomy and Biology, Philadelphia)

Reprinted from

THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. XXXVIII, No. 1, May, 1919

Digitized by the Internet Archive
in 2017 with funding from
This project is made possible by a grant from the Institute of Museum and Library Services as administered by the Pennsylvania Department of Education through the Office of Commonwealth Libraries

https://archive.org/details/relativeabundanc00toya

Reprinted from The Journal of Biological Chemistry, Vol. XXXVIII, No. 1, 1919

RELATIVE ABUNDANCE OF SERUM PROTEINS IN ALBINO RATS AT DIFFERENT AGES.

By IKUZO TOYAMA.

(*From The Wistar Institute of Anatomy and Biology, Philadelphia.*)

(Received for publication, March 6, 1919.)

Wells (1913) studied the relative abundance of serum proteins in rabbits as affected by age as well as by diet. Wells' observation is fundamentally important as the data not only reveal the influence of age, which is much neglected in such studies, but also form the basis for further experimental investigations. It appears desirable that the observation of Wells should be repeated or extended to other laboratory animals which are commonly used. I therefore have made a similar study on the serum of albino rats as these animals are extensively used and furthermore at The Wistar Institute rats from the colony of standard animals can be had for study.

The albino rats used in these experiments were fed on the ordinary laboratory diet and the blood was removed 24 hours after feeding. Suckling rats were isolated from the mother 8 to 16 hours before bleeding. The rats were etherized and bled directly from the carotid artery into a test-tube, care being taken not to sever the trachea or esophagus. The test-tube was closed to prevent evaporation and left at room temperature until the serum had separated. The blood was then centrifuged and the clear serum pipetted off and analyzed. In this work the method devised by Robertson (1915) was used; the total proteins were precipitated by 0.02 N acetic acid and the globulins by ammonium sulfate.

Altogether 66 specimens of serum, secured from 117 albino rats, were classified into fourteen groups according to age. In the majority of specimens, namely in 44, the desired amount of serum (1.5 to 2.0 cc.) was obtained from one rat, while in the younger rats, it was obtained by using from two to seventeen rats at once, the number increasing with decreasing age.

Refractive Index of Serum of Albino Rats at Different Ages.

Hatai (1918) found that the refractive index of the serum of albino rats varies clearly according to their age and in general increases with increasing age. Hatai noticed three distinct phases in the graph. The first phase is represented by the period of suckling, that is about the first 18 days, during which the refractive index rises rapidly. This period of rapid rise is followed by sudden fall of the graph, after which it rises again until the animal is about 70 to 90 days old. The end of this phase just precedes puberty. After 70 to 90 days of age the graph rises very slowly throughout the remainder of the span of life. The period of transition between the end of sexual maturity and the beginning of the adult phase is marked by irregularities in the graph, although no definite interpretation of this phenomenon has been given by Hatai.

For the rats employed in the present investigation I have also determined the refractive index of the serum. The results are given in Table I, Column 5, and also in the upper part of Chart 1.

Despite the fact of the small number (117) of rats examined by me, Chart 1 clearly shows in the upper graph the three phases mentioned by Hatai (1918). In my series, however, the end of both the suckling phase and the end of the puberty phase come several days later than in the records by Hatai; that is, at about 22 days for the former instead of 18 days, as shown by Hatai's rats, and for the latter at 85 instead of 70 days. This difference in the age at which the two critical phases occur is interesting because it is related to a period of poor nutrition in the rat colony in general, although the best animals obtainable were used for this work. It was noted at this time that the young were small for their age and required to be left with the mother longer than usual. The absolute value of the refractive index was also less than that found by Hatai.

Proteins of Blood Serum.

The results of the determinations of the proteins are shown in Table I, and the graphic representation is given in the lower part of Chart 1. A discussion of the changes in relative proportions of the serum proteins, shown in Chart 1, follows.

During the suckling phase, or for about the first 23 days, the percentage of total proteins and also of both albumin and globulins shows an increase with age. Reiss (1909) has also reported a similar increase of protein content in the human serum in the suckling period—after the period of very high content of protein which occurs during the first days of life in man.

From the end of the suckling period (23 days) to the age of 30 days, the percentage of globulins falls rapidly and then rises again

TABLE I.

Changes in Refractive Index as well as in the Proportional Amount of Serum Proteins in Albino Rats at Different Ages.

Age.	No. of samples analyzed.	Body wt. (average).	Body length (average).	N_D	Total proteins.	Albumins.	Globulins.	Non-protein substances.	Relative amount of	
									Albumin	Globulin.
days		*gm.*	*mm.*		*per cent*	*per cent*	*per cent*	*per cent.*		
Newborn.	1	—	—	1.34101	2.8	1.7	1.1	1.6	60.7	39.3
7	1	10.8	—	1.34095	2.9	1.6	1.3	1.5	55.2	44.8
14	2	18.3	82	1.34245	3.9	2.4	1.5	1.2	61.5	38.5
22	2	26.5	97	1.34399	4.4	2.3	2.1	1.6	52.3	47.7
30	5	24.7	96	1.34350	4.7	3.5	1.2	1.5	74.5	25.5
50	5	51.1	115	1.34508	5.0	2.8	2.2	1.5	56.0	44.0
80	12	80.8	144	1.34648	5.7	3.3	2.4	1.5	57.9	42.1
87	5	69.4	142	1.34694	6.2	4.0	2.2	1.3	64.5	35.5
94	7	76.8	146	1.34647	5.9	3.7	2.2	1.4	62.7	37.3
103	9	78.5	142	1.34745	6.2	3.8	2.4	1.5	61.3	38.7
140	4	130.8	171	1.34722	5.9	3.1	2.8	1.5	52.5	47.5
180	3	129.0	172	1.34700	6.3	4.2	2.1	1.3	66.7	33.3
305	6	177.9	191	1.34876	6.5	2.9	3.6	1.7	44.6	55.4
385	4	197.8	196	1.34699	6.0	2.7	3.3	1.4	45.0	55.0

until 50 days. The percentage of albumins, however, shows at first an increase and then a decrease, in opposition to the concentration of globulins. During this period of alteration in the proportion of globulin to albumin in the serum, the value for total proteins of the serum does not show any corresponding fluctuation, but is rising continuously. It is known that young rats do not take any large quantity of milk after 23 days of age, but probably solid food only. Wells (1913) found that milk-fed animals show

an increase in the relative amount of the globulins over the control series fed on a mixed diet. The alteration shown in the period between 22 and 30 days may therefore be due to the fact that the rat becomes accustomed to solid food so that the composition of the blood is slowly restored, and the ratios which the various proteins bore to the total protein before the change in diet reappear.

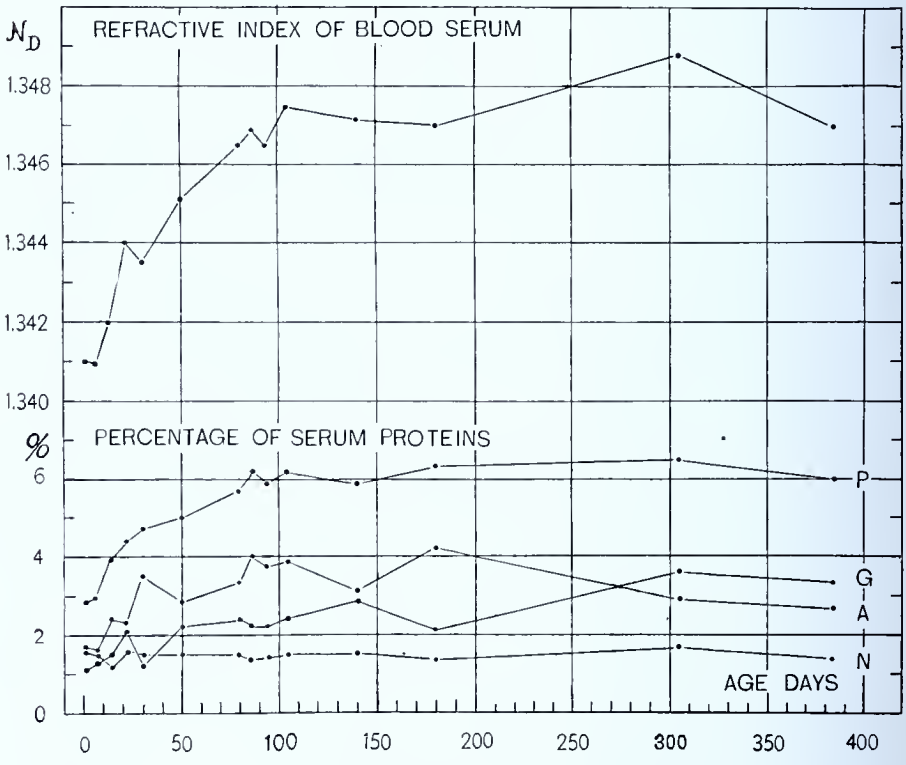

Chart 1. Changes in the refractive index and in the relative amount of serum proteins, based on the data given in Table I.

P = protein. G = globulin.
A = albumin. N = non-protein.

After adjustment to the change in diet the percentage of globulins and the percentage of albumins show a gradual increase with age from 50 to about 90 days. From puberty on the subsequent alteration in the percentage of total proteins is small, though it continues to rise throughout the rest of life. Between 80 to 180 days of age the relative proportion of globulins to albumins shows some irregularity, probably due to high individual variations.

Despite the fact of the irregularities just mentioned there is strong indication that the relative amount of albumin gradually decreases, while that of globulin gradually increases throughout the rest of life so far as examined. We thus find that at the age of about 275 days the ratio of globulin to albumin becomes the reverse of that found at an earlier age.

It should be stated that we always made careful postmortem examinations, and the blood of only those animals which were free from lung infection, as well as any other noticeable pathological alterations, was used for the purpose of analysis.

We infer therefore that the changes in the relative proportion of globulin and of albumin noted in the older rats were not produced by the immediate pathological alterations nor any disease present at the time of killing. It is, however, conceivable that the animals might during their earlier life have suffered from infection, yet so long as we have no direct evidence that the older rats suffered from infectious diseases, I feel justified in making the tentative conclusion that the globulin fraction relative to the albumin fraction shows some increase with advancing age. The percentage of non-protein substances (N) shows no variation corresponding with the age of the rat.

From his observations on rabbits Wells (1913) concludes that the percentage of total proteins increases with age between 21 to 140 days, but that fully adult animals have a slightly lower content.

My observations on the rat, however, show no such tendency of decrease in the content of proteins, even with rats which are 300 days old, although the rats at 385 days do show a slight falling off. Hatai (1918) questions whether or not the adult rabbits used by Wells were entirely normal, and so far as my own data are concerned the rats which were free from visible disease show no indication of falling off, when fully adult and even older. On the contrary, there is a strong tendency for steady increase of the proteins. Wells' data show no decisive increase of the relative amount of globulins at even 1 year of age, while the rats 300 days old give a greater relative amount of globulin than of albumin, thus reversing the relation present at younger periods. I am unable to state whether the serum of rabbits older than 1 year will show similar alterations in the serum proteins as are noted here in the rat.

CONCLUSIONS.

1. The present observation supports the view held by Hatai (1918) that there are in the albino rats three distinct phases in the growth curve of the refractive index of serum; namely, suckling, puberty, and maturity.

2. The percentage of total proteins increases very rapidly during the suckling period, but during the puberty phase growth is slow. The percentage of proteins during the adult period shows only slight increase, but at the age of 385 days a small fall was noticed; this may or may not be significant.

3. The percentage of albumin, though irregular, shows rapid increase for the first 30 days of age. This increase is followed by a rapid fall which soon is followed by a steady rise until the end of sexual maturity. The albumin content at the adult period shows first slow and later a steady relative diminution. The globulin fraction also shows a rapid increase until the end of the suckling period. This rise is followed by a sharp fall and reaches a minimum at the age of 30 days, caused probably by the change of diet from milk to solid food. The relative amount of globulin then shows a steady increase, and at the age of about 275 days the relative amount of globulins becomes greater than that of the albumin.

4. The percentage of the non-protein bodies is practically constant throughout the entire span of life.

BIBLIOGRAPHY.

Hatai, S., The refractive index of the blood serum of the albino rat at different ages, *J. Biol. Chem.*, 1918, xxxv, 527.

Reiss, E., Untersuchungen der Blutkonzentration des Säuglings, *Jahrb. Kinderh.*, 1909, lxx, 311.

Robertson, T. B., A micro refractometric method of determining the percentages of globulin and albumin in very small quantities of blood serum, *J. Biol. Chem.*, 1915, xxii, 233.

Wells, C. E., The influence of age and of diet on the relative proportions of serum proteins in rabbits, *J. Biol. Chem.*, 1913, xv, 37.